TITLE: THE EXTRACELLULAR MATRIX OF MONO- AND MIXED-SPECIES CARIOGENIC BIOFILM AT TWO DEVELOPMENTAL PHASES

AUTHORS: Castillo, M.C.[1]; Novais, T.F.[1]; Quivey, R.G.[2]; Klein, M.I.[1]

INSTITUTION: 1. Department of Dental Materials and Prosthodontics, Araraquara Dental School, Univ Estadual Paulista - UNESP, Araraquara, SP, BRAZIL; **2.** Center for Oral Biology, University of Rochester, Rochester, New York, UNITED STATES.

ABSTRACT

The construction of the extracellular matrix (ECM) in cariogenic biofilms is coordinated by *Streptococcus mutans*. The ECM form a 3D scaffold, favoring microbial adhesion and cohesion. Moreover, the ECM provides microenvironments with acidic niches that lead to demineralization of tooth (*i.e.*, caries). *S. mutans* UA159 parental strain or knockout mutant strains mono-species and mixed-species (S. mutans, *Actinomyces naeslundii* ATCC12104 and *Streptococcus gordonii* DL-1) biofilms were grown onto saliva-coated hydroxyapatite discs with culture medium containing saliva + 0.1% sucrose, alternated with saliva + 0.5% sucrose +1% starch. (37°C / 5% CO_2). *S. mutans* knockout mutants of *lytTS* genes (ΔSMU.525 and ΔSMU.526 - eDNA), operon *dltABCD* (ΔSMU.1538 and ΔSMU.1541 – lipoteichoic acid or LTA) and the gene *gtfB* (insoluble exopolysaccharides) were used to modulate the presence of ECM components. The biofilms were processed at 67h and 115h, to assess population (CFU), biomass, total protein and ECM components: eDNA, and water soluble (WSP) and insoluble (ASP) exopolysaccharides. The data were analyzed by ANOVA one-way and Tukey test (α=0.05). In mixed-species biofilms, *S. mutans* showed a significant increase in the CFU numbers from 67h to 115h for all strains ($p<0.05$); while *A. naeslundii* and *S. gordonii* population decreased. *S. mutans* mono-species biofilms showed a different behavior, with an increase only in the CFU for ΔSMU.1538 and ΔSMU.1541. Regarding insoluble biomass and total protein, the data for both mono- and mixed-species biofilms were similar ($p>0.05$), and increased at similar rate over time – exception was a slight decrease in protein for mixed-species containing the *gtfB* mutant eDNA recovered at 67 and 115h demonstrated that the mutants lacking the genes *lytTS* resulted in higher quantity in both mono- and mixed-species biofilms ($p<0.05$ vs. parental and all mutant strains tested), with increase over time. The amount of WSP at both ages was similar for all strains (except for gtfB that had lowest amount), and increased from 67 to 115h. ASP recovered at 67h was more abundant in mono-species biofilms, but at 115h the mixed-species biofilms had more ASP. Moreover, the amount of WSP and ASP were lowest for *gtfB* mono- and mixed-species biofilms, at 67 and 115hs. Thus, the two

developmental phases of mono- and mixed-species biofilms are different, with dynamic increase of ECM components that may ensure the cariogenicity of these biofilms.

Keywords: Dental Biofilm, Dental Caries, Extracellular Matrix, *Streptococcus mutans.*

Development Agencies: *FAPESP* 2014/05423-0 *and* 2014/21355-4.